

LOCATELLI et al.
Serial No. 09/831,820
November 21, 2003

IN THE CLAIMS

Amend the claims as follows.

1. (Currently Amended) A method for the quantitative detection of a target nucleic acid sequence of a HHV-8, nucleic acid (target) from a sample, which comprises the following steps:

a) extraction of the nucleic acid from the sample with another nucleic acid (calibrator) previously added to the sample itself, said calibrator having the same sequence of the target nucleic acid, said calibrator having i) the same sequence of the target nucleic acid, apart from the region hybridizing to a target the probe, which is randomized with respect to the corresponding region of the target nucleic acid, maintaining the same nucleotide composition, and ii) a T_m equal to the target nucleic acid $T_m \pm 4^\circ\text{C}$ with respect to the corresponding region of the target nucleic acid, having the same nucleotide composition, but with a random sequence, and a similar T_m ,

b) mixing the extracted target nucleic acid and calibrator with a forward primer which has the sequence of SEQ ID NO:5, with a reverse primer which has the sequence of SEQ ID NO:6, primers (forward and reverse) annealing to the corresponding regions on the calibrator and on the target nucleic acid, with said target probe probes bearing a reporter and a quencher, said target probe having the sequence of SEQ ID NO:7, and a calibrator probe bearing a reporter and a quencher, said calibrator probe having a sequence of SEQ ID NO:8, and annealing said forward primer, said reverse primer, and said probes bearing a reporter and a quencher to the target nucleic acid and to the corresponding randomized region on the calibrator, and

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~~with a~~ in the presence of nucleic acid polymerase with 5' -3' nuclease activity, under in
suitable conditions to carry out a polymerization reaction, and

c) determination of the signal associated with the reporters released due to the
5' polymerase nuclease activity.

2. (Currently Amended) A method for the quantitative detection of a target
nucleic acid sequence of a HHV-8, nucleic acid (target) from a sample, which comprises
the following steps:

a) extraction of the nucleic acid from the sample with another nucleic acid
(calibrator) previously added to the sample itself, said calibrator ~~having the same~~
~~sequence of the target nucleic acid, said calibrator~~ having i) the same sequence as the
target nucleic acid, apart from the regions hybridizing to ~~a~~ the probe or to the primers,
which are randomized with respect to the corresponding regions of the target nucleic
acid, maintaining the same nucleotide composition, and ii) a T_m equal to the target
nucleic acid $T_m \pm 4^\circ\text{C}$

b) mixing the extracted target nucleic acid and calibrator a forward primer which
has the sequence of SEQ ID NO:5, with a reverse primer which has the sequence of
SEQ ID NO:6, with primers (forward and reverse) annealing to the target nucleic acid
and to the corresponding randomized regions on the calibrator, with a probe probes
bearing a reporter and a quencher, said target probe having the sequence of SEQ ID
NO:7, and a calibrator probe bearing a reporter and a quencher, said calibrator probe
having a sequence of SEQ ID NO:8, and annealing said forward primer, said reverse
primer, and said probes bearing a reporter and a quencher to the target nucleic acid

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and to the corresponding randomized region on the calibrator, ~~and with a~~ in the presence of nucleic acid polymerase with 5' -3' nuclease activity, ~~under in suitable~~ conditions to carry out a polymerization reaction, and

c) determination of the signal associated with the reporters released due to the 5' polymerase nuclease activity.

3. (Previously Amended) Method according to the claim 1, wherein the calibrator T_m is comprised in the $\pm 4^\circ\text{C}$ range of the target nucleic acid T_m .

4. (Previously Amended) Method according to claim 1 wherein the 5' end of the probes is 1 to 30 nucleotides from the 3' end of the forward primer.

5. (Currently Amended) Method according to claim 1, wherein the probe is ~~probes have the~~ 3' end blocked in order to prevent the extension by the polymerase.

6. (Currently Amended) Method according to claim 1, wherein ~~said nucleic acids, said probes and said primers are DNA sequences, and the~~ nucleic acid polymerase is thermostable DNA polymerase with 5' -3' nuclease activity.

Claims 7 and 8 (Canceled).

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9. (Currently Amended) Method according to claim 1, wherein said ~~probe~~ ~~probes~~ ~~include~~ includes a quencher label able to reduce or to avoid the reporter label fluorescence when the ~~probes are~~ probe is free in solution.

Claims 10-18 (Canceled).